



Green synthesis of silver nanoparticles using *Swertia chirayita* leaves and its effect against selected human pathogens

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Abstract

Many herbal medicinal plants were used in folk medicine to treat the variety of diseases and illnesses. In yesteryears the herbal medicinal plants were applied or employed as a treatment without knowing about the active compounds present in it which is responsible to cure the disease. With the advancement in research, many of those active compounds were identified that could explain the effective use of such plants. Green synthesis of silver nanoparticles using herbal medicinal plants is now known to have antimicrobial activities.

The silver nanoparticles (AgNPs) synthesized by the bio reduction of silver nitrate (AgNO₃) using aqueous leaf extracts of plants *Swertia chirayita* were characterized using UV-Visible spectrophotometer and its antibacterial activity was studied by agar well diffusion method against different pathogenic bacteria. The antibacterial study shows good zone of inhibition against human pathogenic bacteria *E.coli* and *Klebsiella* species and hence it proves to have potential application in the area of nano-medicine such as drug delivery, antibacterial, antifungal and antiviral activities, novel drugs, etc. involving the development and application of materials and technologies in nanometer length scales.

Keywords: medicinal plants, green synthesis, silver nanoparticles, antimicrobial activity

Introduction

Medicinal plants referred to as herbal medicines by World Health Organization in the year 2008 are prepared from raw plant materials such as leaves, roots, shoots, buds, etc. either by extraction, fractionation, purification, concentration or by any physical or biological methods. Globally, the extract of plants has been used for antibacterial, antifungal and antiviral activities and shows several therapeutic applications [1-6].

Green synthesis of nanoparticles is an eco-friendly, clean approach for preparation of nanoparticles where chemicals used are renewable, non-toxic and reducing agents. The silver nanoparticles prepared using plant leaf extract are effective against various types of microorganisms and has multi-disciplinary applications in the field of health sciences, bio-sensors and drug-delivery systems [7-14].

Swertia chirayita plant belongs to family of Gentianeaceae and generally available in India, Nepal and China at an altitude of 1200-3000m. It is an important medicinal plant and a valuable bitter tonic and their preparation has been extensively used as antimicrobial, antifungal activity, anti-diarrhoeal, anti-helminthic, anti-inflammatory, anti-leucorrhoea, anti-rheumatic and anticancer anti-oxidative etc. in prevention and treatment of variety of diseases for its pharmacological properties. It helps in lowering the blood glucose levels and generally consumed by people with type-II diabetes. Flavonoids, anthro-quinones, xanthonons, phenolic acids saponins, tanins, terpenoids and tocopherols have many important roles in human nutrition as antioxidants [15-19]. By using different leaf extracts of *Swertia chirayita*, silver nanoparticles (AgNPs) are synthesized and its phytochemical and antimicrobial activities were studied in this present experiment.

Materials and Methods

Preparation of *Swertia chirayita* leaf extracts

Fresh and healthy leaves of Indian herbal medicinal plants *Swertia chirayita* were collected from North Guwahati hills, Assam, India. The leaves were washed thoroughly with tap water to remove all the dust and unwanted visible adherent particles. The leaves were allowed to dry at room temperature for a week and then kept in hot air oven at 50°C for 24 hours. The dried leaves were ground into the fined powder using electrical grinder. By Soxhlet extraction method, *Swertia Chirayita* leaf extract was prepared using methanol as a solvent (400ml) and leaf powder (40gm) packed in thimbles. The process of extraction was continued until the solvent in siphon tube of extract dropping from the thimble becomes colourless. The filtered leaf extracts were concentrated using rotary vacuum evaporator maintained at 60°C till all the solvent got evaporated. The dried extract was kept in air tight container at 4°C in for further experimentation (Figure 1).



Fig 1: Preparation of *Swertia chirayita* leaf extract

Phytochemical screening of crude extract

Preliminary qualitative phytochemical screenings were carried out for the extracts of *Swertia chirayita* to determine the bioactive compounds as per standard methods [20-21].

Detection of Alkaloids: The formation of a yellow cream precipitate on treatment of leaf extracts dissolved in dilute HCL with a few drops of Mayer's reagent indicates the presence of alkaloids.

Detection of Flavonoids: The formation of an orange colour precipitate on addition of few ml of H₂SO₄ (sulphuric acid) with the sample extracts indicates the presence of flavonoids.

Detection of Steroids: 2ml of extracts were mixed with a few drops of acetic anhydride in the test tube and heated and cooled followed by addition of conc. H₂SO₄ along the side of the test tube carefully. Appearance of a brown ring at the junction of two layers with the upper layer turning green indicates the presence of steroids.

Detection of Terpenoids: The appearance of reddish brown colour precipitate on treating 2ml of leaf extract dissolved in 2ml of chloroform with 2ml of concentrated sulphuric acid followed by heating for about 2 minutes indicates the presence of terpenoids.

Detection of Phenols: The formation of bluish black colour precipitate on addition of few drops of 5% ferric chloride to leaf extracts indicates the presence of phenols.

Detection of Saponins: The appearance of a creamy stable persistent of small bubbles or foams in a test tube while shaking and warming a mixture of 5ml of leaf extracts with 5ml of distilled water indicates the presence of saponins.

Detection of Tannins: The formation of a green precipitate on addition of few drops of Ferric Chloride solution to 2ml of leaf extracts dissolved in distilled water indicates the presence of tannins.

Detection of Carbohydrates: The formation of reddish brown precipitate on heating the leaf extract dissolved in distilled water (hydrolysed with dilute hydrochloric acid, neutralized with alkali) with Fehling's solution A and B indicates the presence of carbohydrates

Detection of Oils and Resins: The development of transparent appearance on filter paper while applying leaf extracts on filter paper indicates the presence of oils and resins.

Bio-synthesis of Silver Nanoparticles

The *Swertia Chirayita* leaf extract was added to silver nitrate (AgNO₃) solution and stirred continuously maintaining at an ambient temperature (15°C to 25°C) for green synthesis of silver nanoparticles (AgNPs). The AgNPs synthesis was monitored at different time interval using varying concentration of silver nitrate (AgNO₃) and leaf extracts (Figure 2). The whole experiment was carried out under dark condition.

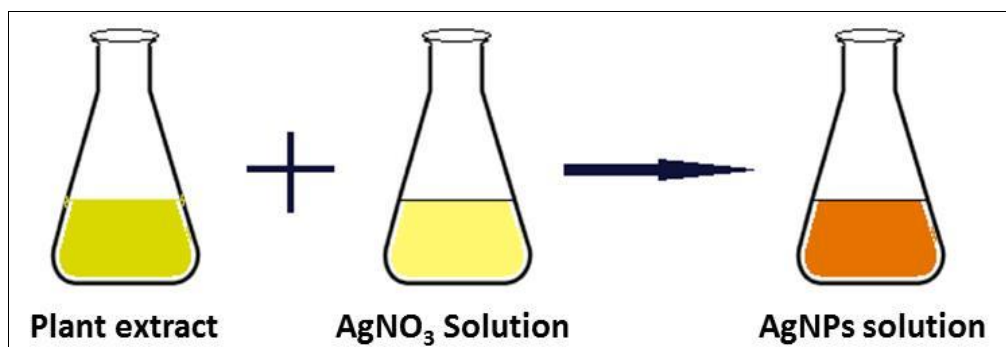


Fig 2: Green synthesis of Silver nanoparticles using *Swertia chirayita* leaves extracts

Characterization of Synthesized Silver Nanoparticles

Double Beam UV-visible spectrophotometer, Make and Model Shimadzu UV-1601 of wavelength range 200nm-800nm with a resolution of 1nm was used to study the bio-reductive property of *Swertia chirayita* leaf extracts and formation of AgNPs. Optical Density (O.D) was recorded to confirm the reduction of silver nitrate.

Assessment of Antimicrobial efficacy of Silver Nanoparticles

The antimicrobial efficacy of silver nanoparticles against the human pathogenic microorganisms *Escherichia coli* and *Klebsiella* species were carried out by well-diffusion methods. Fresh overnight cultures of each pathogenic strain (bacteria 100 μ L) were taken and spread evenly on the nutrient agar plates using sterile cotton swabs. With the help of gel puncture 6mm wells were made on the agar plates and 50 μ L of the silver nanoparticle and leaves extracts was poured into the each well with a micropipette. For further growth the agar plates were incubated at 37 $^{\circ}$ C for 24 hour in incubator. After inoculation, the diameter of zone of inhibition around each well was measured. For control, 50 μ L of 1mM silver nitrate (AgNO₃) solutions was used.

Results and Discussion

Phytochemical investigation

The phytochemical investigation of *Swertia chirayita* leaf extract is shown in Table 1:

Table 1: Phytochemical analysis for *Swertia chirayita*

Phytochemicals/ Test Name	Observations	Results
Alkaloids		
1. Mayer's Test	Cream colour	+
Flavonoids		
1. H ₂ SO ₄ Test	Orange colour precipitate	+
Steroids		
1. Leibermann Burchard Test	Violet to Blue or Green colour formation	+
Terpenoids		
1. Salkowski Test	Reddish brown precipitate	+
Phenols		
1. Ferric chloride Test	Deep Blue to Black colour formation	+
Saponin		
1. Saponin test	Stable persistence of small bubbles	+
Tannins		
1. Ferric Chloride Test	Brownish green/blue black colour	+
Carbohydrates		
1. Fehling's Test	Red brownish colour precipitate	+
Oils & Resins		
1. Spot test	Filter Paper Methods	--

+ indicates presence

- indicates absence

UV-Visible spectral analysis of silver nanoparticles

The silver nanoparticles (AgNPs) synthesized from *Swertia chirayita* leaves extracts and Silver Nitrate solution (AgNO₃) were characterized by using UV-Visible spectrophotometer showing absorbance peak in the range of 430-448 nm. Reaction of the leaf extracts with silver nitrate solution was monitored at different time intervals and parallel changes in colour were observed. The reaction was carried out under dark condition in order to avoid photo-activation of silver nitrate at room temperature. The initial colour of the AgNO₃ solution on treatment with *Swertia chirayita* leaf extracts was light yellow. After 24 hours the colour turned pale yellow, finally the colour changed to dark brown during a period of 96 hours (Figure 3). The breakdown of silver ions

and formation of silver nanoparticles in the reaction mixture is confirmed by colour change. Reduction of Ag^+ to Ag^0 is confirmed by the colour change of solution from colourless to brown due to excitation of Surface Plasmon Resonance (SPR) indicating a very characteristic property of silver nanoparticles formation. On the other hand, no colour change was observed in the control test of 1mM AgNO_3 solution. The absorbance peak of silver nanoparticles (AgNPs) was measured at different time intervals for varying concentration of AgNO_3 (1-5mM) with constant leaf extracts (100 μL) constant and vice-versa by varying the leaf extract solution (1-500 μL) with constant AgNO_3 (1mM). The intensity of absorption peaks increases with increase in the concentration of the AgNO_3 solution and leaf extract concentration (Table 2 and Figure 4).

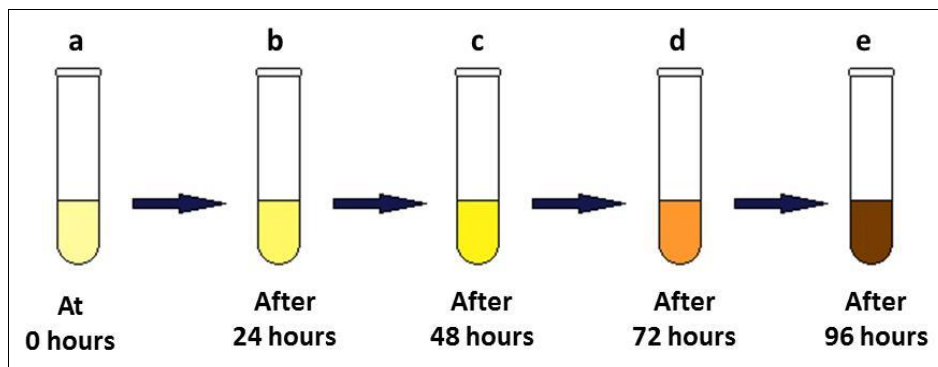


Fig 3: The colour of *Swertia chirayita* leaf extract treated with AgNO_3 (1-5mM) (a) At 0 hours; (b) After 24 hours; (c) After 48 hours; (d) After 72hours; (e) After 96 hours

Table 2: Absorbance measured by varying the concentration of *Swertia chirayita* leaf extract keeping AgNO_3 concentration constant and varying concentration of AgNO_3 solution keeping *Swertia chirayita* leaf extract concentration constant

Sl.No.	Conc. of Leaf extract	Conc. Of AgNO_3	OD at 0 Hours	OD at 24 Hours	OD at 48 Hours	OD at 72 Hours	OD at 96 Hours
A. With varying Conc. of leaf extract (100 μL -500 μL) keeping AgNO_3 (1mM) constant	Control 0 μL	1mM	0.000	0.000	0.000	0.000	0.000
	100 μL	1mM	0.077	0.148	0.228	0.358	0.397
	200 μL	1mM	0.081	0.152	0.236	0.362	0.401
	300 μL	1mM	0.088	0.167	0.247	0.368	0.411
	400 μL	1mM	0.089	0.174	0.251	0.372	0.418
B. With varying Conc. of AgNO_3 (1-5mM) keeping Leaf Extract (100 μL) constant	100 μL	Control 0mM	0.000	0.000	0.000	0.000	0.000
	100 μL	1mM	0.138	0.365	0.505	0.601	0.68
	100 μL	2mM	0.139	0.592	0.62	0.859	0.94
	100 μL	3mM	0.148	0.725	0.897	1.037	1.147
	100 μL	4mM	0.155	0.744	0.943	1.094	1.229
	100 μL	5mM	0.168	0.765	1.102	1.123	1.254

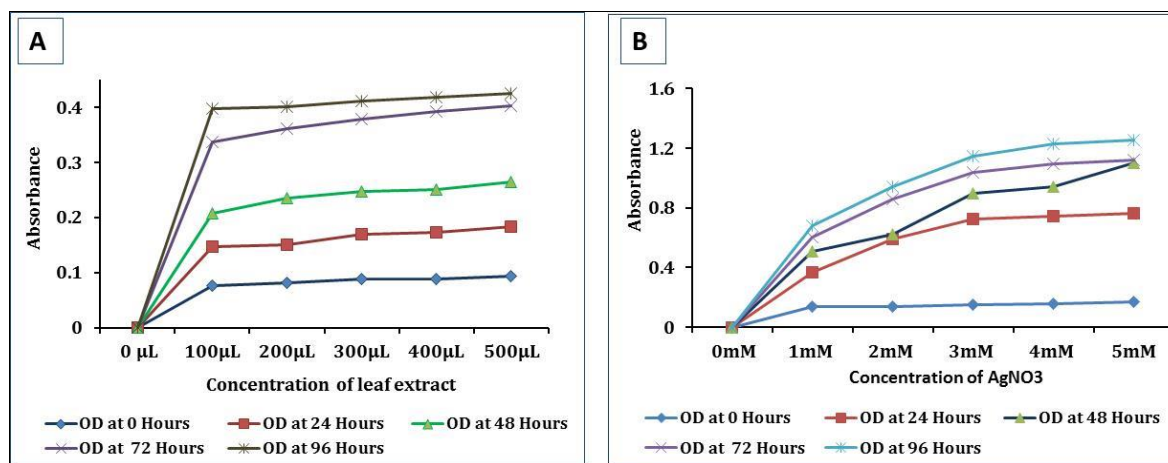


Fig 4: Bar diagram showing absorbance of silver nanoparticle formation by varying concentration of leaf extracts keeping AgNO_3 concentration constant and varying concentration of AgNO_3 solution keeping leaf extract concentration constant

Antimicrobial activity of silver nanoparticles

The antimicrobial activity of *Swertia chirayita* leaf extract and bio-reduced silver nanoparticles prepared using leaf extract were investigated against two bacterial strains, *E.coli* and *Klebsiella species* using well diffusion method is shown in Table 3 and Figure 5. It is believed that the phytochemical compounds in *Swertia chirayita* leaf extract and the silver ions of AgNPs inhibit growth of micro-organisms by disrupting the cell membrane, cellular proteins, DNA strands hereby affecting the permeability and respiratory functions of the pathogens.

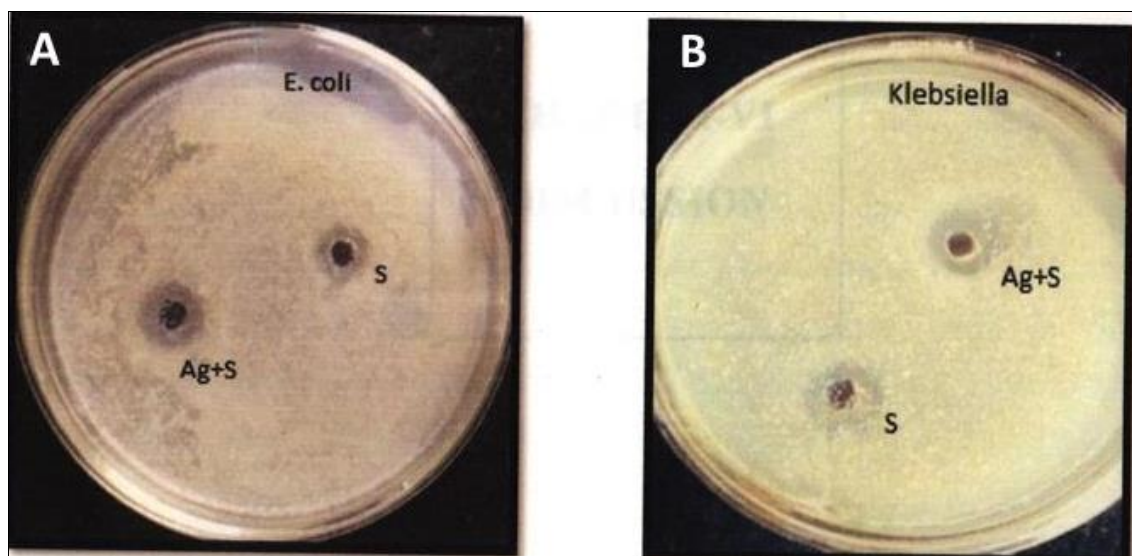


Fig 5: Zone of inhibition against bacterial strains (a) *E.coli*; (b) *Klebsiella* species after 24 hours using *Swertia chirayita* leaf extract and silver nanoparticle

Table 3: Zone of inhibition (in mm) and Bar diagram of *Swertia chirayita* leaf extract and silver nanoparticles against *E. Coli* and *Klebsiella* sp.

Components	<i>E. coli</i>	<i>Klebsiella species</i>
<i>Swertia chirayita</i> leaf extract	9 mm	11 mm
Silver nanoparticles	12 mm	14 mm

Component	<i>E. coli</i> (mm)	<i>Klebsiella species</i> (mm)
<i>Swertia chirayita</i> leaf extract	9	11
Silver nanoparticles	12	14

Conclusion

The green synthesis of AgNPs using *Swertia chirayita* leaf extract is very simple eco-friendly and cost effective method as the synthesis process does not involve any sophisticated equipment and toxic chemicals. The silver nanoparticles prepared using *Swertia chirayita* leaves extracts are non-toxic and shows antibacterial activity against human pathogenic bacteria *E.coli* and *Klebsiella* species. It has wide application in the field of bio-sensors, health science, drug delivery systems, etc. The synthesized silver nanoparticles (AgNPs) exhibited maximum absorbance peak in the range of 430-448 nm. The findings of the present study lay the foundation for future large scale prospective studies from North East India. Also it adds scientific merit to long term use of *Swertia chirayita* as traditional herbal medicines.

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Conflict of interest

None

Financial Disclosure

None to disclose

References

1. Aleem A, Kabir H. Review on swertia chirata as traditional uses to its phytochemistry and pharmacological activity. *Journal of Drug Delivery and Therapeutics*,2018;8(5-s):73-8.
2. Bajaj S, Singh V, Ali M. New triterpenic compounds from Swertia chirata. *Ancient Science of Life*,2017;37(1):37.
3. Rónavári A, Igaz N, Adamecz DI, Szerencsés B, Molnar C, Kónya Z, Pfeiffer I, Kiricsi M. Green silver and gold nanoparticles: Biological synthesis approaches and potentials for biomedical applications. *Molecules*,2021;26(4):844.
4. Khan LU, Khan RA, Khan S, Bano SA, Fasim F, Uzair B. Phytochemical screening and assessment of pharmacological properties of Swertia chirayita (Roxb. ex Fleming) root methanolic extract.
5. Kumari G, Guleria A, Kaur J. Phytochemical and Thin Layer Chromatographic Evaluation of Swertia chirayita Buch.-Hams. Ex Wall at Different Developmental Stages. *International Journal of Current Microbiology and Applied Sciences*,2019;8(02):855-68.
6. Shukla JK, Dhakal P, Uniyal RC, Paul N, Sahoo D. Ex-situ cultivation at lower altitude and evaluation of Swertia chirayita, a critically endangered medicinal plant of Sikkim Himalayan region, India. *South African Journal of Botany*,2017;109:138-45.
7. Singha S, Neog K, Kalita PP, Talukdar N, Sarma MP. Biological synthesis of silver nanoparticles by Neptunia oleraceae. *International Journal of Basic and Applied Biology*,2014;2(2):55-9.
8. Pradhan BK, Badola HK. Swertia chirayita, a threatened high-value medicinal herb: Microhabitats and conservation challenges in Sikkim Himalaya, India. *Mountain Research and Development*,2015;35(4):374-81.
9. Padhan JK, Kumar V, Sood H, Singh TR, Chauhan RS. Contents of therapeutic metabolites in Swertia chirayita correlate with the expression profiles of multiple genes in corresponding biosynthesis pathways. *Phytochemistry*,2015;116:38-47.
10. Patil K, Dhande S, Kadam V. Therapeutic Swertia chirata-an overview. *Research Journal of Pharmacognosy and Phytochemistry*,2013;5(4):199-207.
11. Sharma N, Pandey R, Gowthami R. *In vitro* conservation and cryopreservation of threatened medicinal plants of India. In *Conservation and Utilization of Threatened Medicinal Plants* Springer, Cham, 2020, 181-228.
12. Das P, Medhi OK. Spectroscopic and Electrochemical Studies on Cobaloximes and Alkylcobaloximes in Aqueous Surfactant Micelles as Models of Vitamin B12. *Journal of Surface Science and Technology*,2011;27(3):211.
13. Singh RL, Singh P, Agarwal A. Chemical constituents and bio-pharmacological activities of Swertia chirata: a review. *NPAIJ*,2012;8(6):238-47.
14. Subedi I, Karki TB. Phytochemical and Antimicrobial Screening of native plant Swertia chirayita (Roxb. ex Fleming) karst from Rasuwa district of Nepal. *Journal of Tropical Life Science*,2018;8(2):260576.
15. Afzal A, Aftab B, Siddique J, Babar S, Sohail A, Yasir M, Hanif S. Phytochemical and antimicrobial activity analysis of swertia chirayita and artemisia absinthium plant extracts. *Biological and Clinical Sciences Research Journal*, 2021, 2021(1).
16. Khan LU, Khan RA, Khan S, Bano SA, Fasim F, Uzair B. Phytochemical screening and assessment of pharmacological properties of Swertia chirayita (Roxb. ex Fleming) root methanolic extract.
17. Vailanka MS, Nayak K, & Sheeba, E. Anticancer activity of medicinal plant swertia chirata. *International Journal of Current Research*,2015:19860-19862.
18. Woo SY, Win NN, Oo WM, Ngwe H, Ito T, Abe I, Morita H. Viral protein R inhibitors from Swertia chirata of Myanmar. *Journal of bioscience and bioengineering*,2019;128(4):445-9.
19. Parveen B, Parveen A, Parveen R, Ahmad S, Ahmad M, Iqbal M. Challenges and opportunities for traditional herbal medicine today with special reference to its status in India. *Annals of Phytomedicine*,2020;9(2):97-112.
20. Harborne AJ. *Phytochemical methods a guide to modern techniques of plant analysis*. Springer science & business media, 1998, 30.
21. Raaman N, *Phytochemical Techniques*. India: New India Publishing Agency, India, 2006.